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Genetics

Control of gene expression by a mobile recombinational switch

(inversion/homologous recombination/transposon/kanamycin resistance)

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ABSTRACT **Transposable recombinational switches may play important roles in the evolution of bacterial populations by increasing flexibility in the control of expression of particular genes and thereby maintaining heterogeneity in clones of cells growing in a uniform environment. Experiments reported here show that Tn*5*-112, a deletion derivative of kanamycin-resistance transposon Tn*5*, can function as such a mobile recombinational switch. The internal deletion in Tn*5*-112 removes transcription termination signals and permits transcription initiated within the element to continue into nearby bacterial genes. Consequently, in one orientation Tn*5*-112 stimulates distal gene expression, whereas in the other orientation the normal polarity imposed by wild-type Tn*5* intervenes and distal gene expression is not stimulated. Because Tn*5*-112 contains terminal inverted repeats, intramolecular recombination can invert the Tn*5*-112 element and alter gene expression. Tn*5*-112 is transposition deficient. Its mobility derives from the recessive nature of the transposition deficiency and, in this study, from the possibility of homologous recombination which permits its placement in either orientation at any site occupied by another Tn*5* element.**

The first demonstration of transposable elements came from analyses of mutable loci in maize. Discrete genetic elements that could move from site to site were recognized as agents that induced chromosome aberrations and that modified the activity of genes near their sites of insertion in a developmentally regulated fashion. It was suggested that such elements might also control the activities of many genes during normal development (1, 2).

The many recently discovered bacterial transposons (3, 4) led to the intriguing evolutionary concept of genome plasticity—that genes can be gained, lost, or shifted to new positions by processes that do not require extensive nucleotide sequence homology. The potential involvement of transposons in the evolution of new regulatory systems is also apparent: These elements create polar mutations which block transcription of genes in an operon distal to their insertion sites (3). Two transposons (IS2 and Tn3) contain promoters that stimulate the expression of distal genes when the elements are inserted in the correct orientation (5, 6). One transposon (Tn5) stimulates distal gene expression from a fraction of its sites of insertion, independently of its orientation, as if new promoters are created by the fusion of Tn5 with certain target sequences (7).

Many transposons contain long terminal inverted repetitions. Because a crossover between inverted repeats inverts interstitial unique sequences (Fig. 1), the first discovery of such segments (8) suggested possibilities of additional flexibility in gene regulation by recombinational switching. Two such naturally occurring recombinational switches have been discovered. One, in *Salmonella*, determines which of two alternative flagellar antigens will be synthesized (9, 10); the other, in phages Mu and P1, determines which of two alternative host range specificities

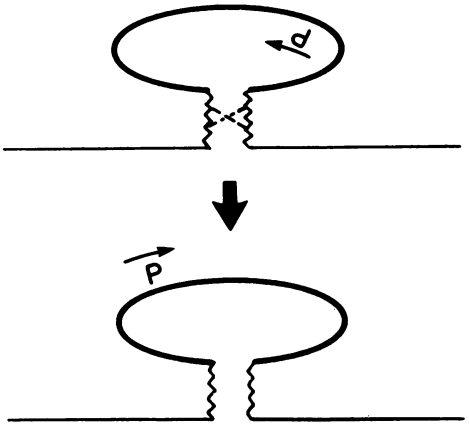

FIG. 1. Inversion by crossing over between inverted repeats.

the progeny viruses will exhibit (11, 12). The fact that specific inversion functions are encoded by genes in or near the invertible segment in each of these two switches (ref. 11; M. Simon, personal communication) attests to the importance of the flexibility these switches provide. It seems likely that the phage host range switch is part of or derived from a transposable element because the homologous invertible segments in phages Mu and P1 are flanked by nonhomologous DNA sequences (11).

I report here that Tn5-112, a simple deletion derivative of the kanamycin-resistance transposon Tn5, has gained the ability to act as a recombinational switch which turns on distal gene expression from an internal promoter in one orientation but not in the other. By homologous recombination one can both place Tn5-112 in either orientation at any site occupied by another Tn5 element and can invert Tn5-112 after placing it in a particular genomic site. I anticipate that Tn5-112 will prove useful as a model for the evolution of recombinational switches and the mechanism of intramolecular exchange.

MATERIALS AND METHODS

The *Escherichia coli* K-12 bacterial and λ phage strains used are listed in Table 1 and 2. A map of transposon Tn5 and its derivatives is shown in Fig. 2. General procedures for phage growth and crosses, bacterial crosses, plasmid DNA extractions, restriction endonuclease digestions, transformation of competent cells, assays of β-galactosidase, and the minimal and complex media used have been described (7, 18).

RESULTS

Tn*5*-112 Can Stimulate Nearby Gene Expression. Tn5-112 was derived from transposon Tn5 by the deletion of an internal 3-kilobase (kb) segment (17). The deletion leaves a terminal 186-base-pair (bp) inverted repeat and brings the kanamycin resistance gene (*kan*^{r}) near the right end of the element as

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Abbreviations: kb, kilobase(s); bp, base pair.

Table 1. Bacterial strains

	Genotype	Ref. or origin
DB1506	F′*proB*⁺*lac*⁺/Δ*proB-lac* Δ*trpE5*	7
DB1506-124	F′*proB*⁺*lacZ*124::Tn*5*/Δ*proB-lac* Δ*trpE5*	This work*
DB1506-132	F′*proB*⁺*lacZ*132::Tn*5*/Δ*proB-lac* Δ*trpE5*	This work*
DB1506-155	F′*proB*⁺*lacZ*155::Tn*5*/Δ*proB-lac* Δ*trpE5*	This work*
DB1506-204	F′*proB*⁺*lacP*204::Tn*5*/Δ*proB-lac* Δ*trpE5*	This work*
DB1506-217	F′*proB*⁺*lacZ*217::Tn*5*/Δ*proB-lac* Δ*trpE5*	This work*
DB1544	F⁻Δ*proB-lac ara str* [pMC4/S10]†	13

* P1 transduction of DB1506-0 using phage grown on *lac*::Tn*5* insertion mutants of DB1446 (7).

† The pMC4 plasmid consists of the Tet^r ColE1 derivative pMB9 ligated to a 10-kilobase (kb) *Eco*RI fragment containing most of the 3-kb *lacZ* gene, *lacI*, and 7 kb of lambdoid phage sequences adjacent to *lacI*. It is *lacZ*⁻ because the *Eco*RI fragment is missing the last 50 base pairs of *lacZ* (13).

drawn in Fig. 2. *Kan*^{r} is transcribed left to right (17). It seemed possible that signals present in wild-type Tn*5* which stop the *kan*^{r} transcript from continuing into nearby bacterial genes might be deleted in Tn*5*-112. If this were the case, placement of Tn*5*-112 in an operon in the proper orientation would stimulate transcription of distal genes. This was tested first by placing Tn*5*-112 in the promoter region of the *lac* operon, thereby permitting analysis of its effects on gene expression by assays of β-galactosidase.

Strains carrying Tn*5*-112 in both orientations at a site in the *lac* promoter designated *lacP*204 (7) were generated in two steps, by recombination *in vivo* between Tn*5* elements present at *lacP*204 and in infecting lambdoid phages. First, wild-type Tn*5* present at *lacP*204 was replaced by Tn*5*-410 (Trp⁺) brought into the cell in a λ phage vector as outlined in Fig. 3. Then, in an analogous cross the Tn*5*-410 element at the *lacP* site was replaced by Tn*5*-112 (Kan^r). One isolate from each of 33 independent replacements of Tn*5*-410 by Tn*5*-112 was tested for *lac* operon expression on MacConkey agar. Sixteen isolates formed pale pink colonies, indistinguishable from isogeneic *lacP*204::Tn*5*-wild type strains, and 17 isolates formed dark pink colonies, indicative of much stronger lactose fermentation (Table 3).

β-Galactosidase assays confirmed the existence of two classes of Tn*5*-112 replacements at site *P*204 (Table 3). The strains designated "OFF," like the ancestral wild-type Tn*5* strain, made no detectable β-galactosidase in the absence of a *lac* operon inducer. In contrast, the mutants designated "ON" synthesized β-galactosidase constitutively at a rate which, fortuitously, was close to the basal level of expression of a wild-type *lac* operon.

Table 2. Phage strains

Phage	Transduces	Ref. or origin
λ*b*221 *c*26	—	14
λ*b*221 *c*I857 Tn*5*	Kan^r	15
λ*b*221 *c*I857 Tn*5*-131	Tet^r	7
λ*b*221 *c*I857 Tn*5*-410	TrpE⁺	7
λ*b*221 *c*I857 Tn*5*-135	Tet^r	7
λ*b*221 *c*I857 Tn*5*-137	Tet^r	7
λ*b*221 *c*I857 Tn*5*-112	Kan^r	This work*

* λ*b*221 *c*I857 Tn*5*-112 was generated by recombination *in vivo* between λ*b*221 *c*I857 Tn*5*-410 and ColE1::Tn*5*-112 as described (7).

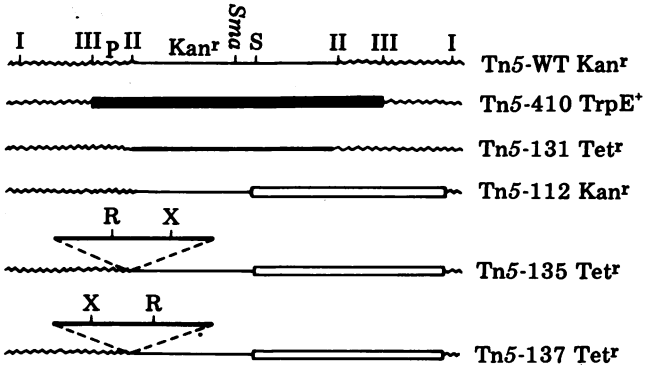

FIG. 2. Map of Tn*5* and its derivatives. Wavy lines, inverted repetitions; P, promoter for expression of kanamycin resistance. Restriction endonuclease cleavage sites: I, *Hpa* I; II, *Bgl* II; III, *Hin*dIII; S, *Sal* I; *Sma*, *Sma* I; R, *Eco*RI; X, *Xba* I. Tn*5*-410 contains a 5300-base-pair fragment containing the *trp* operon *E* and *D* genes in place of the central *Hin*dIII fragment of wild-type Tn*5* (16). Tn*5*-131 contains a 2700-base-pair *Bgl* I fragment from Tn*10* encoding tetracycline resistance (Tet^r) as a substitution which replaced the central *Bgl* II fragment of wild-type Tn*5*. Tn*5*-112 contains a deletion extending from the *Sal* I site to the *Hpa* I site 186 base pairs from the end of the right component of Tn*5*'s inverted repeat which renders this element defective in transposition. In Tn*5*-135 and Tn*5*-137, a fragment conferring Tet^r generated by *Bgl* II digestion of Tn*10* was inserted into the left component of the inverted repeat of Tn*5*-112 in opposite orientations to generate Tn*5*-135 and Tn*5*-137. These elements confer Tet^r but not Kan^r, apparently because the *tet*^{r} insert is polar on *kan*^{r} (17).

To test the generality of the effect of Tn*5*-112 seen in the *lac* promoter, similar replacements were carried out at four sites in *lacZ*. Approximately half of the replacements resulted in enhanced *lacY* expression (the ON phenotype) and half did not (the OFF phenotype) (Table 4).

ON and OFF Phenotypes Depend on the Orientation of Tn*5*-112. Restriction endonuclease analyses were used to test whether the ON and OFF phenotypes depend on the orientation of Tn*5*-112, as predicted by the view that it is the *kan*^{r} transcript which continues into distal bacterial genes. Four independent ON and four independent OFF *lac*::Tn*5*-112 alleles at each of two sites (*lacP*204 and *lacZ*124) were recom-

Table 3. Expression of *lacZ* in cultures of *lacP*204::Tn*5* insertion mutants

	Lactose fermentation*	β-Galactosidase†	Phenotype designation	Orientation‡
Bacterial strain:				
Lac⁺	+++	1	Lac⁺	
*lacZ*124::Tn*5*	0	<0.05	LacZ⁻	
*lacP*204::Tn*5*-wild type:				
Orientation I	+	<0.05	OFF	I
Orientation II	+	<0.05	OFF	II
*lacP*204::Tn*5*-112:				
1	++	0.75	ON	II
2	++	0.78	ON	II
3	+	<0.05	OFF	I
4	+	<0.05	OFF	I

The *lac*⁺ bacterial strain was DB1506. Other strains in this table were derived from it by introduction of the indicated Tn*5* element.

* Lactose fermentation was scored on MacConkey agar.

† β-Galactosidase was assayed as described (18). The values are expressed relative to the basal (noninduced) level for *lac*⁺ strain DB1506 = 1.

‡ The orientation was determined from restriction endonuclease digestion as shown in Figs. 3 and 4.

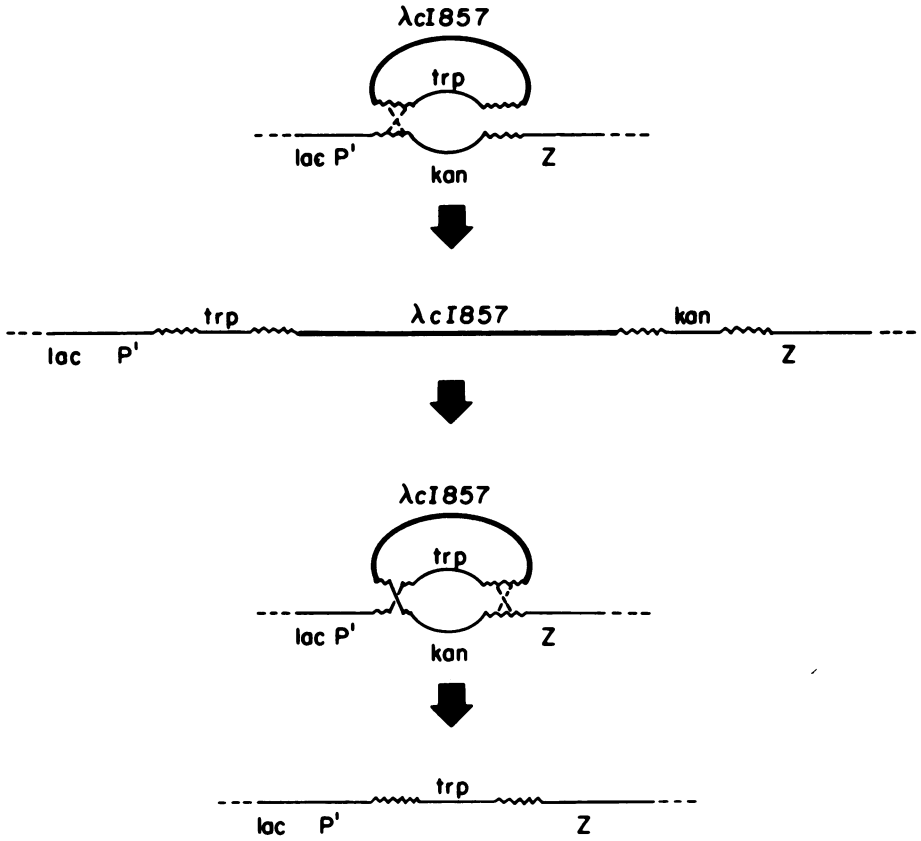

FIG. 3. Replacement of Tn5 (Kan^r). Shown here is the replacement of wild-type Tn5 (Kan^r) in the *lac* promoter by Tn5-410(Trp$^+$). After infection of Trp$^-$ cells containing Tn5 with λ*b*221 cI857, Tn5-410 lysogens (line 2) are selected as Trp$^+$ λ immune colonies at 32°C. Because the *b*221 deletion makes this phage unable to integrate into the bacterial attachment site, stable lysogens generally result from a crossover between homologous portions of inverted repetitions of wild-type Tn5 and Tn5-410 present in phage λ. These lysogens were selected by their Trp$^+$ immune phenotype at 32°C. Nonlysogenic segregants were selected by their ability to grow at 41°C, a temperature that induces λ*c*I857 prophage development. The heat-resistant Trp$^+$ colonies, found at frequencies of 10^{-2}–10^{-3}, arose by a second crossover on the other side of the inhomology between wild-type Tn5 and Tn5-410. To ensure a high yield of pure Trp$^+$ Kan^s clones, the lysogenic cultures were routinely grown for 5–10 generations in tryptophan-free media containing glucose which inhibits adsorption of λ phage particles. Analogous procedures resulted in the replacement of wild-type Tn5 by Tn5-131 (Tet^r) and then replacement of Tn5-410 or Tn5-131 by Tn5-112 (Kan^r). The Trp$^+$ Kan^s and Trp$^-$ Kan^r nonlysogenic segregants from the Tn5–λTn5-410 lysogens generated in the first step shown were obtained at equal frequencies. In contrast, the Trp$^+$ Kan^s class of recombinants from the Tn5-410–λTn5-112 lysogens generated in the following step were at least 100-fold more frequent than the desired Kan^r Trp$^-$ class. The low frequency of recombinants that retain Tn5-112 probably reflect the different sizes of the two regions in which Tn5-410 and Tn5-112 are homologous (1200 bp vs. 186 bp; see Fig. 1) and the preference for crossing over in the larger (1200 bp) region.

bined into the colE1*lac* plasmid pMC4 *in vivo*, and the recombinant plasmids were analyzed by digestion with *Sma* I, an enzyme that cleaves Tn5-112 once and the pMC4 plasmid twice. For Tn5-112 insertions at a given site, one pattern of restriction fragments was associated with the ON phenotype in each case and a second pattern was associated with the OFF phenotype (Fig. 4). These results indicate that the ON phenotype is produced when Tn5-112 is oriented such that transcription of *kan*^{r} proceeds toward distal *lac* operon genes.

The *Kan*^{r} Promoter Is Implicated in the ON Phenotype. Further evidence indicating that readthrough transcription from the *kan*^{r} promoter is responsible for the ON phenotype comes from replacement of Tn5-112 in the ON orientation with derivatives in which a *tet*^{r} segment inserted between the *kan*^{r} gene and its promoter blocks *kan*^{r} transcription (Tn5-135 and Tn5-137 in Fig. 2). Each of 20 independent replacements of Tn5-112 analyzed, 10 by Tn5-135 and 10 by Tn5-137, resulted in loss of the ON phenotype.

Inversion of a Tn*5*-112 Element in Place in the Bacterial Genome. To extend the view of Tn5-112 as a mobile recombinational switch, I tested the ability of the element inserted in *lacZ* to change *lacY* expression by an intramolecular exchange resulting in inversion (Fig. 1). Derivatives of strains carrying Tn5-112 at site *lacZ*155 in the OFF orientation and able to grow on minimal melibiose agar (LacY$^+$) were selected at 41°C. They were recovered at a frequency of approximately 10^{-7}; 12% (10 of 83 tested) were Kan^s and thus resulted from excision of the element. The majority (88%), however, were Kan^r, a phenotype expected from inversion of Tn5-112. To test whether these isolates did result from inversion of Tn5-112, nine independent ON alleles were recombined into the pMC4 plasmid and analyzed by *Sma* I digestion as described above. The sizes of fragments of each of the nine ON isolates were identical and indicated that the element had inverted at its site of insertion (Fig. 5).

Because the 186-bp inverted repeats of Tn5-112 are probably near the lower size limit for homologous pairing in generalized recombination (20), it seemed possible that the inversion detected here is actually a sequence-specific event dependent on a function analogous to the inversion functions of the *Salmonella* phase variation or phage host range switches. Such a site-specific inversion function might act in the absence of the *recA* gene product which is necessary for intermolecular homologous recombination. To test this prediction, I also selected LacY$^+$ Kan^r derivatives of *lacZ*155::Tn5-112 OFF mutants in a *recA*$^-$ background ($\approx 10^{-7}$). The LacY$^+$ Kan^r derivatives

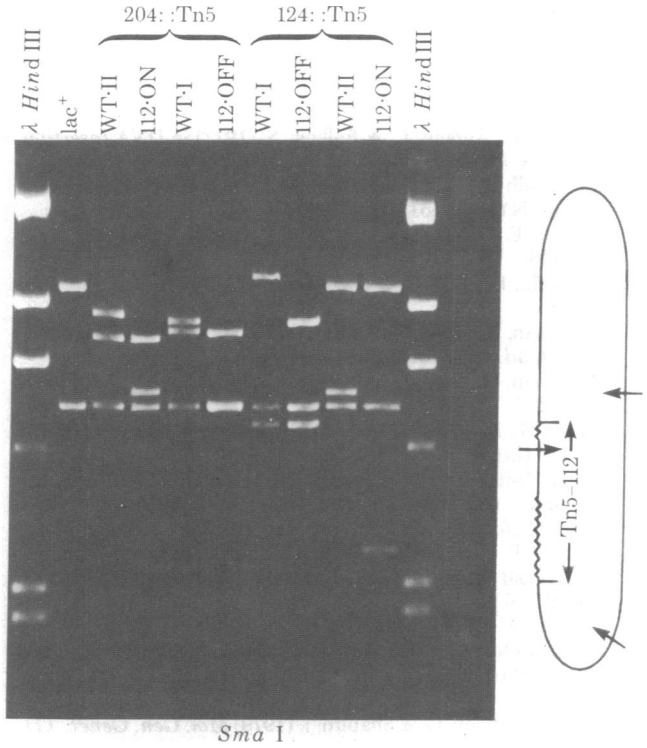

FIG. 4. Agarose gel electrophoresis of *Sma* I digests of pMC4::Tn*5*-112 and pMC4::Tn*5*-wild type DNAs. The numbers 204 and 124 refer to insertion sites *P*204 (in the *lac* promoter) and *Z*124 (near the carboxy terminus of *lacZ*). lac$^+$ designates the parental pMC4 plasmid not containing Tn*5*. Diagrammed at the right are *Sma* I cleavages of a pMC4::Tn*5*-112 plasmid. The fragment sizes in the λ *Hin*dIII reference digests are (from top to bottom) 23, 9.8, 6.6, 4.5, 2.5, and 2.2 kb (19). From these standards, the following estimates of fragment sizes (in kb) were obtained: pMC4 lac$^+$, 11 and 5.5 (common to all plasmids analyzed); fusion fragments resulting from insertion into the 11-kb pMC4 fragment, 11.2 and 2.9, and 8.6 and 5.0 for *lacZ*124::Tn*5*-112 ON and OFF, respectively, 11.2 and 5.9, and 12.0 and 5.0 for *lacZ*124::Tn*5* wild-type II and I, respectively, 8.1 and 5.9, and 5.5 and 8.8 for *lacP*204::Tn*5*-112 ON and OFF, respectively, and 8.1 and 9.6, and 8.4 and 8.8 for *lacP*204::Tn*5* wild-type II and I, respectively.

occurred with equal frequencies in *recA*$^+$ and *recA*$^-$ strains, which would indicate that Tn*5*-112 inversion is *recA*$^+$ independent. However, further analyses (to be presented elsewhere) indicate a *recA*$^+$ dependence of part of the normal inversion process because the process in *recA*$^-$ cells resulted in a major DNA sequence rearrangement.

DISCUSSION

Transposons increase the plasticity of the content and organization of an organism's genome; switching mechanisms cause variable expression of particular genes in uniform environments. The selective advantage of switching mechanisms becomes clear when considering that bacteria sometimes form large populations in which most or all individuals are derived clonally from a single ancestor—for example, during invasive infection by a bacterial pathogen. For *Salmonella*, the ability of small numbers of cells in an otherwise homogeneous clone to express new sets of antigens decreases the ease with which the host's immunological defense can destroy the invading bacteria. Similar considerations indicate that the ability of phages Mu and P1 to infect totally different bacterial hosts after induction of a lysogen enhances their reproductive potential.

The first metastable switch described in prokaryotes was a

Table 4. LacY phenotypes resulting from replacement by Tn*5*-112

Insertion site*	Number†	
	ON	OFF
*lacP*204	17	16
*lacZ*124	13	21
*lacZ*132	8	9
*lacZ*155	6	9
*lacZ*217	10	7
Total	54	62

* The strains carrying Tn*5*-112 at selected sites were generated as described in Fig. 2 and the text by using insertions of Tn*5*-410 at sites *P*204 and *Z*124 and of Tn*5*-131 at sites *Z*132, *Z*155, and *Z*217.

† *lacY* expression was scored on MacConkey melibiose plates at 41°C; fermenting colonies are pink or red and nonfermenting colonies are white. With the exception of lac*Z*124::Tn*5*-112 isolates, ON indicates melibiose fermentation, and OFF indicates no fermentation. In the case of insertions in site *Z*124, both wild-type Tn*5* and Tn*5*-112 OFF permit weak expression of *lacY* (7). The ON isolates comprise one uniform class exhibiting higher levels of *lacY* expression.

physiological switch. In bacteriophage λ, two different repressor proteins (the products of genes *cI* and *cro*) compete for the chance to act on a single operator region. Once a critical threshold concentration of either protein is achieved, its binding to the common operator blocks synthesis of the other (21, 22).

Recombinational switches have also evolved and may be more common than physiological switches. In these, inversion by intramolecular exchange creates or disrupts a transcriptional linkage between a particular gene and its promoter. In the cases of the invertible segments that control host range of phages Mu and P1, and that control flagellar antigen type in *Salmonella*, the invertible segment is linked to genes whose products specifically catalyze inversion (9–12).

The incorporation of such recombinational switches into transposons would represent an important evolutionary ad-

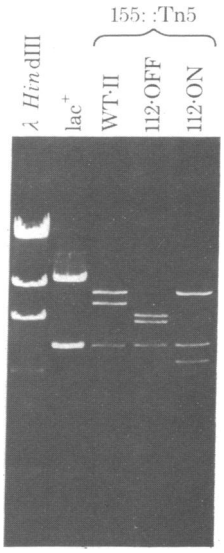

FIG. 5. Restriction endonuclease analyses showing reversal of Tn*5*-112 orientation due to selection for change of OFF to ON phenotype. The *lacZ*155::Tn*5*-112 and Tn*5* wild-type alleles were recombined into pMC4 plasmids, digested with *Sma* I, and electrophoresed as described in the text and in Fig. 4. The approximate sizes of fusion fragments (in kb) were: 9.2 and 8.2 for Tn*5* wild type, 7.1 and 6.6 for Tn*5*-112 OFF, and 9.2 and 4.8 for Tn*5*-112 ON.

vance. A switch evolved to service one set of genes could modulate the expression of other sets of genes simply by moving to appropriate new sites.

The results presented here show that Tn5-112 is such a mobile recombinational switch. Because termination signals present in wild-type Tn5 are deleted in Tn5-112, transcription initiated within Tn5-112 can lead to expression of neighboring genes. Although Tn5-112 is transposition deficient, this defect is recessive. In addition, by homologous recombination Tn5-112 can move to any site to which wild-type Tn5 may previously have transposed. Once Tn5-112 is in place, a crossover between its inverted repeats reverses the orientation of the element and can place a different set of genes under the control of the element's promoter. From analyses to date it appears that, although the complete inversion process as outlined in Fig. 1 requires the bacterial generalized recombination system, key steps sufficient to change the OFF phenotype to ON occur in *recA*$^-$ cells. Experiments are needed to provide a more precise description of the product of the *recA*-independent reaction, and a better understanding of the inversion process—its possible dependence on a site-specific recombination function and its partial independence of the *recA* function which is necessary for homologous exchange between different DNA molecules.

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